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Applicants: Nam, H.-J., and S.-H. Kim

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04/08/00-KOR

Search Strategy

FILE 'USPATFULL' ENTERED AT 14:29:44 ON 09 DEC 2003 ✓

L1 E NAM HYUK JUN/IN
 E KIM SANG HERN/IN
 3 S E2
 E KIM SANG-HERN/IN

FILE 'WPIDS' ENTERED AT 14:30:52 ON 09 DEC 2003

L2 E NAM H J/IN
 53 S E3
L3 2 S L2 AND (NC OR NUCLEOCAPSID OR P7)

FILE 'MEDLINE' ENTERED AT 14:32:08 ON 09 DEC 2003

L4 E NAM H J/AU
 8 S E3
 E NAM HYUK JUN/AU
 E KIM S H/AU
L5 1406 S E3
L6 0 S L5 AND (NC OR NUCLEOCAPSID OR P7)
 E KIM SANG-HERN/AU
 E KIM SANG HERN/AU
L7 1 S E3

FILE 'USPATFULL' ENTERED AT 15:23:36 ON 09 DEC 2003

L1 29456 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L2 9423 S L1 AND (NC OR NUCLEOCAPSID OR P7 OR GAG)
L3 3665 S L2 AND (P7 OR NC OR NUCLEOCAPSID)
L4 828 S L3 AND (PSI OR PACKAGING SIGNAL)
L5 506 S L4 AND (EXPRESSION VECTOR?)
L6 358 S L5 AND (GALACTOSIDASE)
L7 13 S L6 AND (NC/CLM OR P7/CLM OR NUCLEOCAPSID/CLM)
L8 28 S L5 AND (NC/CLM OR P7/CLM OR NUCLEOCAPSID/CLM)
L9 15 S L8 NOT L7
L10 5396 S L1 AND PACKAGING
L11 1028 S L10 AND (NC OR P7 OR NUCLEOCAPSID)
L12 854 S L11 AND (ANTIVIRAL? OR SCREENING)
L13 443 S L12 AND (PACKAGING SIGNAL OR PSI)
L14 394 S L13 AND (E. COLI OR MICROORGANISM)
L15 22 S L14 AND AY=1999
L16 9 S L14 AND AY=1998
L17 13 S L14 AND AY=1997
L18 8 S L14 AND AY=1996
L19 47 S L14 AND AY<1996

FILE 'WPIDS' ENTERED AT 15:44:13 ON 09 DEC 2003

L20 16878 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L21 619 S L20 AND (NC OR NUCLEOCAPSID OR P7 OR GAG)
L22 164 S L21 AND (P7 OR NC OR NUCLEOCAPSID)
L23 10 S L22 AND (PACKAGING OR PACKAGING SIGNAL OR PSI)

FILE 'MEDLINE' ENTERED AT 15:59:57 ON 09 DEC 2003

 E BUECHTER D/AU
L24 17 S E3-E6
L25 136279 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L26 629 S L25 AND (NC OR NUCLEOCAPSID OR P7)
L27 96 S L26 AND (PACKAGING OR PACKAGING SIGNAL OR PSI)

L3 ANSWER 1 OF 2 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
AN 2003-785678 [74] WPIDS
DNC C2003-216336
TI RNAs capable of binding with nucleocapsid protein of HIV-1.
DC B04 D16
IN JEONG, S J; KIM, S J; NAM, H J; YOU, J C
PA (AVIX-N) AVIXGEN INC; (JEON-I) JEONG S J; (YOUJ-I) YOU J C
CYC 1
PI KR 2003018483 A 20030306 (200374)* 1p
ADT KR 2003018483 A KR 2001-52594 20010829
PRAI KR 2001-52594 20010829

AB KR2003018483 A UPAB: 20031117
NOVELTY - RNAs capable of binding with a nucleocapsid protein of HIV-1(human immunodeficiency virus-1) are new. The RNAs inhibit the function of nucleocapsid protein and the growth of HIV-1. The RNAs can be useful for prevention and treatment of HIV.
DETAILED DESCRIPTION - The RNAs capable of binding with the nucleocapsid protein of HIV-1 comprise RNA SE8-4, RNA SE10-3, RNA SE10-12, RNA SE10-7, RNA SE8-6, RNA SE10-1, RNA SE10-8, RNA SE8-11, RNA SE8-10, RNA SE10-2, RNA SE10-11, RNA SE8-7, RNA SE8-13, RNA SE10-9 and RNA SE10-4, all comprising fully defined nucleotide sequences, given in the specification.
Dwg.0/10

L3 ANSWER 2 OF 2 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
AN 2002-025900 [03] WPIDS
DNC C2002-007230
TI Novel microorganism for screening HIV inhibitors, is cotransformed with plasmid vector (PV) containing gene expressing HIV nucleocapsid protein and PV containing HIV psi gene and beta-galactosidase reporter gene.
DC B04 D16
IN KIM, S; NAM, H; KIM, S H; NAM, H J; YOU, J C; YOO, J C
PA (YOUJ-I) YOU J C; (YOUJ-I) YOU J; (YOOJ-I) YOO J C
CYC 94
PI WO 2001077312 A1 20011018 (200203)* EN 35p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ
NL OA PT SD SE SL SZ TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM
DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KZ LC LK
LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG
SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
KR 2001051661 A 20010625 (200203)
AU 2000079681 A 20011023 (200213)
KR 2001090959 A 20011022 (200221)
GB 2366291 A 20020306 (200224)
CN 1354790 A 20020619 (200263)
KR 360275 B 20021113 (200330)
JP 2003530105 W 20031014 (200368) 34p
ADT WO 2001077312 A1 WO 2000-KR1173 20001018; KR 2001051661 A Div ex KR
2000-18489 20000408, KR 2000-67294 20001113; AU 2000079681 A AU 2000-79681
20001018; KR 2001090959 A KR 2000-18489 20000408; GB 2366291 A WO
2000-KR1173 20001018, GB 2001-29825 20011213; CN 1354790 A CN 2000-808632
20001018; KR 360275 B KR 2000-18489 20000408; JP 2003530105 W WO
2000-KR1173 20001018, JP 2001-575166 20001018
FDT AU 2000079681 A Based on WO 2001077312; GB 2366291 A Based on WO
2001077312; KR 360275 B Previous Publ. KR 2001090959; JP 2003530105 W
Based on WO 2001077312
PRAI KR 2000-18489 20000408; KR 2000-67294 20001113

AB WO 200177312 A UPAB: 20030513
NOVELTY - A microorganism (I), cotransformed with a plasmid vector containing a gene expressing human immunodeficiency virus (HIV) nucleocapsid protein, and a plasmid vector containing HIV psi gene and beta -galactosidase reporter gene, is new.
USE - (I) is useful for screening HIV packaging inhibitors by culturing (I), treating (I) with putative compounds or compositions of HIV inhibitors, and measuring the degree of change in beta -galactosidase expression in the culture. (I) is useful for high throughput screening (HTS) of HIV inhibitors (claimed).
ADVANTAGE - HIV inhibitors are screened conveniently, effectively and in a simple way, using (I).
Dwg.0/4

L23 ANSWER 3 OF 10 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
AN 2003-646042 [61] WPIDS
DNC C2003-176758
TI Method of determining whether a compound inhibits formation of complex between HIV nucleocapsid protein 7 polypeptide and HIV psi-site oligonucleotide by comparing amount of complex formed in presence/absence of compound.
DC B04 D16
IN BUECHTER, D; HOU, X; MARLOR, C W; RICE, W G; YANG, W; BEUCHTER, D
PA (BUEC-I) BUECHTER D; (HOUX-I) HOU X; (MARL-I) MARLOR C W; (RICE-I) RICE W G; (YANG-I) YANG W; (ACHI-N) ACHILLION PHARM INC
CYC 102
PI WO 2003060098 A2 20030724 (200361)* EN 105p
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS
LU MC MW MZ NL OA PT SD SE SI SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR
KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT
RO RU SC SD SE SG SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA
ZM ZW
US 2003198648 A1 20031023 (200370)
ADT WO 2003060098 A2 WO 2003-US801 20030110; US 2003198648 A1 Provisional US
2002-347369P 20020111, US 2003-339217 20030109
PRAI US 2002-347369P 20020111; US 2003-339217 20030109

AB WO2003060098 A UPAB: 20030923
NOVELTY - Method of determining whether a compound (I) inhibits formation of a complex between HIV nucleocapsid protein 7 (NCp7) polypeptide (II) and an HIV- psi -site oligonucleotide (III) by adding (III) to admixture of (II) and (I) and comparing amount of complex formed with that formed in the absence of (I), where a decrease in amount of complex formed in presence of (I) indicates that (I) inhibits complex formation.
DETAILED DESCRIPTION - Method of determining whether a compound (I) inhibits the formation of a complex between HIV nucleocapsid protein 7 (NCp7) polypeptide (II) and an HIV psi -site oligonucleotide (III) by admixing (II) with (I); adding (III) to the admixture of (II) and (I) under appropriate binding conditions to form (II)-(III) complex; and comparing the amount of complex formed with the amount of complex formed in the absence of (I), thus determining whether (I) inhibits the complex formation, where a decrease in the amount of complex formed in the presence of (I) indicates that (I) inhibits complex formation.
INDEPENDENT CLAIMS are also included for the following:
(1) inhibiting HIV viral replication in a subject by administering (I); and
(2) a kit for detection of agents for use in treatment of HIV

comprising (II), an oligonucleotide capable of binding to (II) under appropriate binding conditions and instructions describing how to create the appropriate binding conditions.

ACTIVITY - Anti-HIV.

MECHANISM OF ACTION - Inhibitor of binding of NCp7 and HIV psi -site oligonucleotide; HIV replication inhibitor; Nucleocapsid-protein-7-Antagonist.

No supporting data given.

USE - For determining whether a compound inhibits formation of complex between (II) and (III). The compounds identified are useful for treating a subject infected with human immunodeficiency virus (HIV) by administering (I) to the subject (claimed).

DESCRIPTION OF DRAWING(S) - The figure shows the result of binding of biotinylated-SL3 to plate immobilized NCp7 proteins.
Dwg.1/7

[NC and psi on separate vectors capable of stably integrating]

L7 ANSWER 8 OF 13 USPATFULL on STN
2002:300803 Novel lentiviral packaging cells.
Leboulch, Philippe, Charlestown, MA, UNITED STATES
Westerman, Karen, Reading, MA, UNITED STATES
Genetix Pharmaceuticals, Inc.
US 2002168346 A1 20021114
APPLICATION: US 2002-112894 A1 20020401 (10)
PRIORITY: US 1998-85283P 19980513 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Novel packaging cell lines which produce recombinant retrovirus, free of detectable helper-virus are disclosed. Also disclosed are methods of making the cell lines and methods of producing recombinant retroviruses from the cell lines. Retroviruses produced by the cell lines include lentiviruses, such as HIV, capable of transferring heterologous DNA to a wide range of non-dividing cells. The packaging cells contain at least three vectors which collectively encode retroviral gag, pol, and env proteins, wherein the gag and pol genes are separated, in part, onto two or more different vectors. This is made possible by fusing Vpr or Vpx to pol proteins separated from gag so that the proteins are targeted to assembling virions. Among other advantages, the packaging cells provide the benefit of increased safety when used in human gene therapy by virtually eliminating the possibility of molecular recombination leading to production of replication-competent helper virus.

CLM What is claimed is:

1. A retroviral packaging cell line comprising: a first vector encoding a retroviral gag polyprotein and a portion of a retroviral pol polyprotein; a second vector encoding the remainder of the retroviral pol polyprotein not encoded by said first vector, fused to a Vpr or a Vpx protein or peptide; and a third vector encoding a viral env protein.
2. The packaging cell line of claim 1 wherein said retroviral polyproteins are lentiviral polyproteins.
3. The packaging cell line of claim 2 wherein the lentivirus is selected from the group consisting of HIV-1, HIV-2, SIV, FIV and EIV.
4. The packaging cell line of claim 1 wherein said retroviral gag polyprotein encoded by said first vector comprises retroviral matrix, capsid and nucleocapsid proteins.
5. The packaging cell line of claim 1 wherein said portion of said pol polyprotein encoded by said first vector comprises a retroviral protease.
6. The packaging cell line of claim 1 wherein said remainder of said retroviral pol polyprotein encoded by said second vector comprises a retroviral reverse transcriptase and a retroviral integrase.
7. The packaging cell line of claim 6 wherein said remainder of said retroviral pol polyprotein further comprises a protease cleavage site upstream from said reverse transcriptase and said integrase.
8. The packaging cell line of claim 1 wherein said viral env protein

encoded by said third vector comprises an envelope protein selected from a virus selected from the group consisting of a Type C retrovirus, a lentivirus and Vesicular Stomatitis Virus.

9. The packaging cell line of claim 1 wherein transcription of any one of said proteins encoded by said vectors is driven by an inducible promoter.

10. The packaging cell line of claim 1 wherein transcription of any one of said proteins encoded by said vectors is induced by contact of Cre recombinase with one or more Lox sites contained in said vector.

11. The packaging cell line of claim 1 further comprising a fourth vector containing a packaging signal, a viral long terminal repeat (LTR), and a transgene, wherein co-expression of said fourth vector with said first, second, and third vectors, in said packaging cell line, results in the production of a recombinant, helper-free retrovirus containing said transgene.

12. A method of producing a packaging cell line capable of producing a recombinant, helper-free retrovirus, comprising transfecting a host cell with: a first vector encoding a retroviral gag polyprotein and a portion of a retroviral pol polyprotein; a second vector encoding the remainder of the retroviral pol polyprotein not encoded by said first vector, fused to a Vpr or a Vpx protein or peptide; and a third vector encoding a viral env protein.

13. The method of claim 12 wherein any one of said first, second or third vectors comprises an inducible promoter operably linked to a gene encoding said gag polyprotein, said pol polyprotein, said Vpr protein or peptide, said Vpx protein or peptide, or said env protein.

14. The method of claim 13 further comprising the step of contacting said host cell with an agent which induces said promoter.

15. The method of claim 12 wherein said retrovirus is a lentivirus.

16. The method of claim 15 wherein said lentivirus is selected from the group consisting of HIV-1, HIV-2, SIV, FIV and EIV.

17. The method of claim 12 wherein said retroviral gag polyprotein encoded by said first vector comprises lentiviral matrix, capsid and nucleocapsid proteins.

18. The method of claim 12 wherein said wherein said portion of said pol polyprotein encoded by said first vector comprises a retroviral protease.

19. The method of claim 12 wherein said remainder of said retroviral pol polyprotein encoded by said second vector comprises a retroviral reverse transcriptase and a retroviral integrase.

20. The method of claim 13 wherein said viral env protein encoded by said third vector comprises an envelope protein selected from a virus selected from the group consisting of a Type C retrovirus, a lentivirus and Vesicular Stomatitis Virus.

21. A method of producing a recombinant retrovirus comprising transfecting a host cell with: a first vector comprising a retroviral gag gene and a portion of a retroviral pol gene operably linked to a promoter; a second vector comprising the portion of the retroviral

pol gene not encoded by the first vector and a gene encoding all or a portion of a Vpr or a Vpx protein, wherein both of said genes are expressed as a single fusion protein and are operably linked to a promoter; a third vector comprising a viral env gene; and a fourth vector comprising a viral packaging signal, a viral long terminal repeat (LTR), and a transgene operably linked to a promoter; and recovering the recombinant virus.

22. The method of claim 21 wherein any one of said promoters contained within said first, second, third or fourth vectors is inducible.

23. The method of claim 22 further comprising the step of contacting said host cell with an agent which induces said promoter.

24. The method of claim 21 wherein said retrovirus is a lentivirus.

25. The method of claim 24 wherein said lentivirus is HIV.

26. The method of claim 21 wherein said portion of said pol gene contained in said first vector encodes a retroviral protease.

27. The method of claim 21 wherein said remainder of said pol gene contained in said second vector encodes a retroviral reverse transcriptase and a retroviral integrase.

L7 ANSWER 11 OF 13 USPATFULL on STN

2002:69598 Lentiviral packaging cells.

Leboulch, Philippe, Charlestown, MA, United States

Westerman, Karen, Reading, MA, United States

Genetix Pharmaceuticals, Inc., Cambridge, MA, United States (U.S. corporation)

US 6365150 B1 20020402

APPLICATION: US 1999-311684 19990513 (9)

PRIORITY: US 1998-85283P 19980513 (60)

DOCUMENT TYPE: Utility; GRANTED.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Novel packaging cell lines which produce recombinant retrovirus, free of detectable helper-virus are disclosed. Also disclosed are methods of making the cell lines and methods of producing recombinant retroviruses from the cell lines. Retroviruses produced by the cell lines include lentiviruses, such as HIV, capable of transferring heterologous DNA to a wide range of non-dividing cells. The packaging cells contain at least three vectors which collectively encode retroviral gag, pol, and env proteins, wherein the gag and pol genes are separated, in part, onto two or more different vectors. This is made possible by fusing Vpr or Vpx to pol proteins separated from gag so that the proteins are targeted to assembling virions. Among other advantages, the packaging cells provide the benefit of increased safety when used in human gene therapy by virtually eliminating the possibility of molecular recombination leading to production of replication-competant helper virus.

CLM What is claimed is:

1. A retroviral packaging cell line comprising: a first vector comprising a retroviral gag gene and a portion of a retroviral pol gene which overlaps with the gag gene in the natural retrovirus genome; a second vector comprising the remainder of the retroviral pol gene not contained in said first vector, fused to a Vpr or a Vpx gene; and a third vector comprising a viral env gene.

2. The packaging cell line of claim 1 wherein said retrovirus is a lentivirus.
3. The packaging cell line of claim 2 wherein the lentivirus is selected from the group consisting of HIV-1, HIV-2, SIV, FIV and EIV.
4. The packaging cell line of claim 1 wherein said retroviral gag gene encodes retroviral matrix, capsid and nucleocapsid proteins.
5. The packaging cell line of claim 1 wherein said portion of said pol gene within said first vector encodes a retroviral protease.
6. The packaging cell line of claim 1 wherein said remainder of said retroviral pol gene within said second vector encodes a retroviral reverse transcriptase and a retroviral integrase.
7. The packaging cell line of claim 6 wherein said remainder of said retroviral pol gene further comprises a protease cleavage site.
8. The packaging cell line of claim 1 wherein said viral env gene within said third vector encodes an envelope protein from a virus selected from the group consisting of a Type C retrovirus, a lentivirus and Vesicular Stomatitis Virus.
9. The packaging cell line of claim 1 wherein transcription of any one of said genes within said vectors is directed by an inducible promoter.
10. The packaging cell line of claim 1 wherein transcription of any one of said genes within said vectors is induced by interaction of Cre recombinase with one or more Lox sites contained in said vector.
11. The packaging cell line of claim 1 further comprising a fourth vector comprising a packaging signal, a viral long terminal repeat (LTR), and a transgene, wherein co-expression of said fourth vector with said first, second, and third vectors in said packaging cell line, results in the production of a recombinant, helper-free retrovirus containing said transgene.
12. A method of producing a packaging cell line capable of producing a recombinant, helper-free retrovirus, comprising transfecting a host cell with: a first vector comprising a retroviral gag gene and a portion of a retroviral pol gene which overlaps with the gag gene in the natural retroviral genome; a second vector comprising the remainder of the retroviral pol gene contained in said first vector, fused to a Vpr or a Vpx gene or polypeptide; and a third vector comprising a viral env gene.
13. The method of claim 12 wherein transcription of any one of said genes within said vectors is directed by an inducible promoter.
14. The method of claim 13 further comprising the step of contacting said host cell with an agent which induces said promoter.
15. The method of claim 12 wherein said retrovirus is a lentivirus.
16. The method of claim 15 wherein said lentivirus is selected from the group consisting of HIV-1, HIV-2, SIV, FIV and EIV.

17. The method of claim 12 wherein said retroviral gag gene within said first vector encodes lentiviral matrix, capsid and nucleocapsid proteins.
18. The method of claim 12 wherein said portion of said pol gene within said first vector encodes a retroviral protease.
19. The method of claim 12 wherein said remainder of said retroviral pol gene within said second vector encodes a retroviral reverse transcriptase and a retroviral integrase.
20. The method of claim 13 wherein said viral env gene within said third vector encodes an envelope protein from a virus selected from the group consisting of a Type C retrovirus, a lentivirus and Vesicular Stomatitis Virus.
21. A method of producing a recombinant retrovirus comprising transfecting a host cell with: a first vector comprising a retroviral gag gene and a portion of a retroviral pol gene which overlaps with the gag gene in the natural retrovirus genome, operably linked to a promoter; a second vector comprising the portion of the retroviral pol gene not present within the first vector fused to a Vpr or a Vpx gene; a third vector comprising a viral env gene; and a fourth vector comprising a viral packaging signal, a viral long terminal repeat (LTR), and a transgene operably linked to a promoter; and recovering the recombinant virus.
22. The method of claim 21 wherein any one of said promoters contained within said first, second, third or fourth vectors is inducible.
23. The method of claim 22 further comprising the step of contacting said host cell with an agent which induces said promoter.
24. The method of claim 21 wherein said retrovirus is a lentivirus.
25. The method of claim 24 wherein said lentivirus is HIV.
26. The method of claim 21 wherein said portion of said pol gene contained in said first vector encodes a retroviral protease.
27. The method of claim 21 wherein said remainder of said pol gene contained in said second vector encodes a retroviral reverse transcriptase and a retroviral integrase.

L15 ANSWER 1 OF 22 USPATFULL on STN

2003:67554 Lentiviral vectors.

Chang, Lung-Ji, 3102 57th Ter., NW., Gainesville, FL, United States 32606

US 6531123 B1 20030311

APPLICATION: US 1999-318138 19990525 (9)

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PRIORITY: US 1998-86635P 19980526 (60)

DOCUMENT TYPE: Utility; GRANTED.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention contemplates novel lentiviral vectors which exhibit strong promoter activity in human and other cells. Vectors are provided which are packaged efficiently in packaging cells and cell lines to generate high titer recombinant virus stocks. The present invention further relates to HIV vaccines and compositions for gene therapy. In particular, the present invention provides attenuated replication-competent HIV vaccines and replication defective HIV vectors.

CLM What is claimed is:

1. A packaging vector comprising a nucleotide sequence encoding Gag and Pol proteins of a reference lentivirus, said packaging vector differing from said reference lentivirus at least in that (a) its major splice donor site, while functional, differs in sequence from that of said reference lentivirus sufficiently so that said major splice donor site is not a potential site for homologous recombination between said packaging vector and said reference lentivirus, and (b) it lacks a functional major packaging signal, which vector, after introduction into a suitable host cell, is capable of causing such cell, either through expression from said vector alone, or through co-expression from said vector and a second vector providing for expression of a compatible envelope protein, to produce packaging vector particles comprising functional Gag and Pol proteins and having a normal or a pseudotyped envelope, where said particles are free of the RNA form of said packaging vector as a result of (b) above, where said cell, as a result of said expression or co-expression, produces particles encapsulating the RNA form of a transducing vector possessing a compatible and functional packaging signal if said transducing vector is introduced into said cell where said reference lentivirus is selected from the group consisting of HIV-1, HIV-2 and SIV.
2. The packaging vector of claim 1 in which the reference lentivirus is HIV-1.
3. The packaging vector of claim 1 in which the reference lentivirus is HIV-2.
4. The packaging vector of claim 1 in which the reference lentivirus is SIV.
5. The packaging vector of claim 1 which encodes one or more envelope proteins.
6. The packaging vector of claim 1 which does not encode a functional envelope protein.
7. The packaging vector of claim 1 wherein the major splice donor site of said vector differs in sequence from that of any lentivirus major splice donor site sufficiently so that said major splice donor site is not a potential site for homologous recombination between said packaging vector and said lentivirus.
8. The packaging vector of claim 1 which comprises a sequence encoding lentivirus Env proteins.
9. The packaging vector of claim 1 which comprises a sequence encoding the VSV-G envelope protein.
10. The packaging vector of claim 1 which further differs from said reference lentivirus in that at least portions of at least one gene selected from the group consisting of the env, vpr, vif, and vpu genes of said reference lentivirus is or are deleted.
11. The packaging vector of claim 1 which lacks the native primer binding site of said reference lentivirus.
12. The packaging vector of claim 1 which lacks the native polypurine tract of said reference lentivirus.

13. The packaging vector of claim 1 which lacks a functional nef gene.
14. The packaging vector of claim 1 which further differs from said lentivirus in that the 5' LTR has been modified.
15. The packaging vector of claim 1 in which the 5'LTR is a chimera of a lentivirus LTR and a CMV enhancer/promoter.
16. The packaging vector of claim 1 comprises a tat gene and a TAR sequence.
17. The packaging vector of claim 1 which comprises a rev gene and an RRE element.
18. The packaging vector of claim 1 which further differs from the reference lentivirus in that at least a portion of the tat gene and the TAR sequence are deleted.
19. The packaging vector of claim 1 which further differs from the reference lentivirus in that at least a portion of the env gene and the RRE element are deleted.
20. A packaging cell which comprises the packaging vector of claim 1 and is suitable for production of infectious transducing vector particles encapsulating the RNA form of a transducing vector possessing a compatible and functional packaging signal if said transducing vector is introduced into said cell.
21. A method of producing a transducing vector comprising a remedial gene, in the form of infectious particles, which comprises (a) transfecting a cell with a packaging vector according to claim 1, and, if said packaging vector does not itself provide for expression of a compatible envelope protein, a pseudotyping vector which does provide expression, so said cell is capable of producing packaging vector particles, (b) transfecting said cell with a transducing vector comprising said remedial gene, and a functional packaging signal, but which by itself is incapable of causing a cell to produce transducing vector particles, and (c) causing the cell to produce infectious transducing vector particles comprising said transducing vector in RNA form, said Gag and Pol proteins, and said envelope protein.
22. A kit comprising a packaging vector according to claim 1 and a transducing vector comprising a functional and compatible packaging signal, said transducing vector being incapable by itself of causing a cell transfected by said transducing vector to encapsulate the RNA form of said transducing vector into a lentivirus-like particle.
23. The packaging vector of claim 1 in which the major splice donor site is a modified RSV major splice donor site corresponding to the splice donor site included in SEQ ID NO:9 and SEQ ID NO:10.
24. The packaging vector of claim 1 which is vector PHP-1.
25. The packaging vector of claim 7 wherein the major splice donor site of said vector is substantially identical major splice donor site of said vector is substantially identical to the RSV splice donor site.

26. The cell of claim 20, which further comprises a pseudotyping vector.
27. The cell of claim 20 which further comprises a transducing vector which by itself is incapable of coding for expression of infectious transducing vector particles, but which cell, as a result of the expression of genes of said packaging vector, packages the RNA form of said transducing vector into infectious transducing vector particles.
28. The cell of claim 20 where said transducing vector further comprises a remedial gene.
29. The cell of claim 20 wherein packaging is inducible.
30. The cell of claim 20 in which the major packaging signal of said transducing vector is at least 50% identical to the major packaging signal of said reference lentivirus.
31. A method of delivering a remedial gene to target cells which comprises producing the particles by the method of claim 21 and then (d) infecting the target cells with an effective amount of the particles of step (c).
32. The method of claim 31 in which the cells are nondividing cells.
33. The method of claim 31 in which the target cells are cells in a target mammal.
34. The kit of claim 22, said packaging vector comprising a gene encoding a compatible envelope protein.
35. The kit of claim 22, further comprising a pseudotyping vector comprising a gene encoding a non-lentiviral envelope protein incorporatable into said particles.
36. The method of claim 21 in which the major packaging signal of said transducing vector is at least 50% identical to the major packaging signal of said reference lentivirus.
37. The kit of claim 22 in which the major packaging signal of said transducing vector is at least 50% identical to the major packaging signal of said reference lentivirus.

L15 ANSWER 14 OF 22 USPATFULL on STN

2002:69598 Lentiviral packaging cells.

Leboulch, Philippe, Charlestown, MA, United States

Westerman, Karen, Reading, MA, United States

Genetix Pharmaceuticals, Inc., Cambridge, MA, United States (U.S. corporation)

US 6365150 B1 20020402

APPLICATION: US 1999-311684 19990513 (9)

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PRIORITY: US 1998-85283P 19980513 (60)

DOCUMENT TYPE: Utility; GRANTED.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Novel packaging cell lines which produce recombinant retrovirus, free of detectable helper-virus are disclosed. Also

disclosed are methods of making the cell lines and methods of producing recombinant retroviruses from the cell lines. Retroviruses produced by the cell lines include lentiviruses, such as HIV, capable of transferring heterologous DNA to a wide range of non-dividing cells. The packaging cells contain at least three vectors which collectively encode retroviral gag, pol, and env proteins, wherein the gag and pol genes are separated, in part, onto two or more different vectors. This is made possible by fusing Vpr or Vpx to pol proteins separated from gag so that the proteins are targeted to assembling virions. Among other advantages, the packaging cells provide the benefit of increased safety when used in human gene therapy by virtually eliminating the possibility of molecular recombination leading to production of replication-competant helper virus.

CLM What is claimed is:

1. A retroviral packaging cell line comprising: a first vector comprising a retroviral gag gene and a portion of a retroviral pol gene which overlaps with the gag gene in the natural retrovirus genome; a second vector comprising the remainder of the retroviral pol gene not contained in said first vector, fused to a Vpr or a Vpx gene; and a third vector comprising a viral env gene.
2. The packaging cell line of claim 1 wherein said retrovirus is a lentivirus.
3. The packaging cell line of claim 2 wherein the lentivirus is selected from the group consisting of HIV-1, HIV-2, SIV, FIV and EIV.
4. The packaging cell line of claim 1 wherein said retroviral gag gene encodes retroviral matrix, capsid and nucleocapsid proteins.
5. The packaging cell line of claim 1 wherein said portion of said pol gene within said first vector encodes a retroviral protease.
6. The packaging cell line of claim 1 wherein said remainder of said retroviral pol gene within said second vector encodes a retroviral reverse transcriptase and a retroviral integrase.
7. The packaging cell line of claim 6 wherein said remainder of said retroviral pol gene further comprises a protease cleavage site.
8. The packaging cell line of claim 1 wherein said viral env gene within said third vector encodes an envelope protein from a virus selected from the group consisting of a Type C retrovirus, a lentivirus and Vesicular Stomatitis Virus.
9. The packaging cell line of claim 1 wherein transcription of any one of said genes within said vectors is directed by an inducible promoter.
10. The packaging cell line of claim 1 wherein transcription of any one of said genes within said vectors is induced by interaction of Cre recombinase with one or more Lox sites contained in said vector.
11. The packaging cell line of claim 1 further comprising a fourth vector comprising a packaging signal, a viral long terminal repeat (LTR), and a transgene, wherein co-expression of said fourth vector with said first, second, and third vectors in said packaging cell line, results in the production of a recombinant,

helper-free retrovirus containing said transgene.

12. A method of producing a packaging cell line capable of producing a recombinant, helper-free retrovirus, comprising transfecting a host cell with: a first vector comprising a retroviral gag gene and a portion of a retroviral pol gene which overlaps with the gag gene in the natural retroviral genome; a second vector comprising the remainder of the retroviral pol gene contained in said first vector, fused to a Vpr or a Vpx gene or polypeptide; and a third vector comprising a viral env gene.

13. The method of claim 12 wherein transcription of any one of said genes within said vectors is directed by an inducible promoter.

14. The method of claim 13 further comprising the step of contacting said host cell with an agent which induces said promoter.

15. The method of claim 12 wherein said retrovirus is a lentivirus.

16. The method of claim 15 wherein said lentivirus is selected from the group consisting of HIV-1, HIV-2, SIV, FIV and EIV.

17. The method of claim 12 wherein said retroviral gag gene within said first vector encodes lentiviral matrix, capsid and nucleocapsid proteins.

18. The method of claim 12 wherein said portion of said pol gene within said first vector encodes a retroviral protease.

19. The method of claim 12 wherein said remainder of said retroviral pol gene within said second vector encodes a retroviral reverse transcriptase and a retroviral integrase.

20. The method of claim 13 wherein said viral env gene within said third vector encodes an envelope protein from a virus selected from the group consisting of a Type C retrovirus, a lentivirus and Vesicular Stomatitis Virus.

21. A method of producing a recombinant retrovirus comprising transfecting a host cell with: a first vector comprising a retroviral gag gene and a portion of a retroviral pol gene which overlaps with the gag gene in the natural retrovirus genome, operably linked to a promoter; a second vector comprising the portion of the retroviral pol gene not present within the first vector fused to a Vpr or a Vpx gene; a third vector comprising a viral env gene; and a fourth vector comprising a viral packaging signal, a viral long terminal repeat (LTR), and a transgene operably linked to a promoter; and recovering the recombinant virus.

22. The method of claim 21 wherein any one of said promoters contained within said first, second, third or fourth vectors is inducible.

23. The method of claim 22 further comprising the step of contacting said host cell with an agent which induces said promoter.

24. The method of claim 21 wherein said retrovirus is a lentivirus.

25. The method of claim 24 wherein said lentivirus is HIV.

26. The method of claim 21 wherein said portion of said pol gene contained in said first vector encodes a retroviral protease.

27. The method of claim 21 wherein said remainder of said pol gene contained in said second vector encodes a retroviral reverse transcriptase and a retroviral integrase.

L15 ANSWER 19 OF 22 USPATFULL on STN

2001:82513 Compositions and methods for determining anti-viral drug susceptibility and resistance and anti-viral drug screening.

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US 6242187 B1 20010605

APPLICATION: US 1999-371774 19990810 (9)

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PRIORITY: US 1996-10715P 19960129 (60)

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention provides a method for determining susceptibility for an anti-viral drug comprising: (a) introducing a resistance test vector comprising a patient-derived segment and an indicator gene into a host cell; (b) culturing the host cell from (a); (c) measuring expression of the indicator gene in a target host cell; and (d) comparing the expression of the indicator gene from (c) with the expression of the indicator gene measured when steps (a)-(c) are carried out in the absence of the anti-viral drug, wherein a test concentration of the anti-viral drug is present at steps (a)-(c); at steps (b)-(c); or at step (c) This invention also provides a method for determining anti-viral drug resistance in a patient comprising: (a) determining anti-viral drug susceptibility in the patient at a first time using the susceptibility test described above, wherein the patient-derived segment is obtained from the patient at about said time; (b) determining anti-viral drug susceptibility of the same patient at a later time; and (c) comparing the anti-viral drug susceptibilities determined in step (a) and (b), wherein a decrease in anti-viral drug susceptibility at the later time compared to the first time indicates development or progression of anti-viral drug resistance in the patient. This invention also provides a method for evaluating the biological effectiveness of a candidate anti-viral drug compound. Compositions including resistance test vectors comprising a patient-derived segment and an indicator gene and host cells transformed with the resistance test vectors are provided.

CLM What is claimed is:

1. A method for determining susceptibility for an anti-HBV drug comprising: (a) introducing a resistance test vector comprising a patient-derived segment and an indicator gene into a host cell; (b) culturing the host cell from (a); (c) measuring expression of the indicator gene in a target host cell, wherein the expression of the indicator gene is dependent upon the patient-derived segment; and (d) comparing the expression of the indicator gene from (c) with the expression of the indicator gene measured when steps (a)-(c) are carried out in the absence of the anti-HBV drug, wherein a test concentration of the anti-HBV drug is present at steps (a)-(c); at steps (b)-(c); or at step (c).

2. The method of claim 1 wherein the indicator gene is a non-functional indicator gene.

3. The method of claim 1 wherein the indicator gene is a luciferase gene.

4. The method of claim 1 wherein the indicator gene is an E. coli lacz gene.
5. The method of claim 1 wherein the target host cell is a Jurkat cell line.
6. A resistance test vector comprising a HBV patient-derived segment and an indicator gene.
7. The resistance test vector of claim 6 wherein the indicator gene is a functional indicator gene.
8. The resistance test vector of claim 6 wherein the indicator gene is a non-functional indicator gene.
9. The resistance test vector of claim 6 wherein the indicator gene is a luciferase gene.
10. A packaging host cell transfected with a resistance test vector of claim 6.
11. The packaging host cell of claim 10 that is a mammalian host cell.
12. The packaging host cell of claim 10 that is a human host cell.
13. The packaging host cell of claim 10 that is a human embryonic kidney cell.
14. The packaging host cell of claim 10 that is 293 cells.
15. The packaging host cell of claim 10 that is a human hepatoma cell line.
16. The packaging host cell of claim 10 that is HepG2.
17. The packaging host cell of claim 10 that is Huh7.
18. A method for determining susceptibility for an anti-HBV drug comprising: (a) introducing a resistance test vector comprising a patient-derived segment and a nonfunctional indicator gene into a host cell; (b) culturing the host cell from (a); (c) measuring expression of the indicator gene in a target host cell, wherein the expression of the indicator gene is dependent upon the patient-derived segment; and (d) comparing the expression of the indicator gene from (c) with the expression of the indicator gene measured when steps (a)-(c) are carried out in the absence of the anti-HBV drug, wherein a test concentration of the anti-HBV drug is present at steps (a)-(c); at steps (b)-(c); or at step (c).
19. The method of claim 18 wherein the nonfunctional indicator gene comprises a permuted promoter.
20. The method of claim 18 wherein the nonfunctional indicator gene comprises a permuted coding region.
21. The method of claim 18 wherein the nonfunctional indicator gene comprises an inverted intron.

22. The method of claim 18 wherein the host cell and target cell are the same cell.

23. The method of claim 18 wherein the target cell is a human cell.

24. A method for determining anti-HBV drug resistance in a patient comprising: (a) developing a standard curve of drug susceptibility for an anti-HBV drug; (b) determining anti-HBV drug susceptibility in the patient according to the method of claim 1; and (c) comparing the anti-HBV drug susceptibility in step (b) with the standard curve determined in step (a), wherein a decrease in anti-HBV susceptibility indicates development of anti-HBV drug resistance in the patient.

25. A method for determining anti-HBV drug resistance in a patient comprising: (a) developing a standard curve of drug susceptibility for an anti-HBV drug; (b) determining anti-HBV drug susceptibility in the patient according to the method of claim 18; and (c) comparing the anti-HBV drug susceptibility in step (b) with the standard curve determined in step (a), wherein a decrease in anti-HBV susceptibility indicates development of anti-HBV drug resistance in the patient.

26. A method for determining anti-HBV drug resistance in a patient comprising: (a) determining anti-HBV drug susceptibility in the patient at a first time according to the method of claim 1, wherein the patient-derived segment is obtained from the patient at about said time; (b) determining anti-HBV drug susceptibility of the same patient at a later time; and (c) comparing the anti-HBV drug susceptibilities determined in step (a) and (b), wherein a decrease in anti-HBV drug susceptibility at the later time compared to the first time indicates development or progression of anti-viral drug resistance in the patient.

27. A method for determining anti-HBV drug resistance in a patient comprising: (a) determining anti-HBV drug susceptibility in the patient at a first time according to the method of claim 18, wherein the patient-derived segment is obtained from the patient at about said time; (b) determining anti-HBV drug susceptibility of the same patient at a later time; and (c) comparing the anti-HBV drug susceptibilities determined in steps (a) and (b), wherein a decrease in anti-HBV drug susceptibility at the later time compared to the first time indicates development or progression of anti-HBV drug resistance in the patient.

28. The method of claim 1 wherein the resistance test vector comprises DNA encoding C, P, and X.

29. The method of claim 1 wherein the patient-derived segment comprises a P gene.

30. The method of claim 1 wherein the patient-derived segment comprises an HBV gene.

31. The method of claim 1 wherein the patient-derived segment comprises an HBV RT gene.

32. The method of claim 1 wherein the patient-derived segment comprises an HBV DNA polymerase gene.

33. The resistance test vector of claim 6 wherein the patient-derived segment comprises an HBV P gene.

34. A method for evaluating the biological effectiveness of a candidate anti-HBV drug compound comprising: (a) introducing a resistance test

vector comprising a patient-derived segment and an indicator gene into a host cell; (b) culturing the host cell from step (a); (c) measuring expression of the indicator gene in a target host cell, wherein the expression of the indicator gene is dependent upon the patient-derived segment; and (d) comparing the expression of the indicator gene from step (c) with the expression of the indicator gene measured when steps (a)-(c) are carried out in the absence of the candidate anti-HBV drug compound, wherein a test concentration of the candidate anti-HBV drug compound is present at steps (a)-(c) at steps (b)-(c); or at step (c).

35. The method of claim 34 wherein the resistance test vector comprises DNA encoding HBV P protein.

36. The method of claim 34 wherein the patient-derived segment comprises an HBV gene.

37. A method for determining susceptibility for an anti-HBV drug comprising: (a) introducing a resistance test vector comprising a patient-derived segment and an indicator into a host cell; (b) culturing the host cell from (a); (c) measuring the indicator in a target host cell, wherein a change in the indicator is dependent upon the patient-derived segment; and (d) comparing the measurement of the indicator from (c) with the measurement of the indicator when steps (a)-(c) are carried out in the absence of the anti-HBV drug, wherein a test concentration of the anti-viral drug is present at steps (a)-(c); at steps (b)-(c); or at step (c).

38. The method of claim 37 wherein the indicator comprises a DNA structure.

39. The method of claim 37 wherein the indicator comprises a RNA structure.

40. A method for evaluating the biological effectiveness of a candidate anti-HBV drug compound comprising: (a) introducing a resistance test vector comprising a patient-derived segment and an indicator into a (b) culturing the host cell from step (a); (c) measuring the indicator in a target host cell, wherein a change in the indicator is dependent upon the patient-derived segment; and comparing the measurement of the indicator from step(c) with the measurement of the indicator measured when steps (a)-(c) are carried out in the absence of the candidate anti-HBV drug compound, wherein a test concentration of the candidate anti-viral drug compound is present at steps (a)-(c); at steps(b)-(c); or at step (c).

41. The method of claim 40 wherein the indicator comprises a DNA structure.

42. The method of claim 40 wherein the indicator comprises a RNA structure.

L17 ANSWER 6 OF 13 USPATFULL on STN

2001:36659 Cell transformation vector comprising an HIV-2 packaging site nucleic acid and an HIV-1 GAG protein.

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US 6200811 B1 20010313

APPLICATION: US 1997-822516 19970324 (8)

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PRIORITY: US 1996-15555P 19960402 (60)

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB By transducing cells with an HIV-1-MN molecular clone deleted in the major packaging sequence, a stable HIV-1 packaging cell line, .psi.422 was produced. .psi.422 cells form syncytia with CD4 positive cells, correctly express HIV-1 structural proteins, and produce large amount of mature particles with normal RT activity. These particles are not infectious. When stably transfected with an HIV-based retroviral vector, the .psi.422 cell line produces hybrid virions capable of transducing CD4 positive cells with high efficiency (e.g., 10.sup.5 cells/ml). The availability of this stable, noninfectious HIV-1 packaging cell line capable of generating high titer HIV vectors enables the use of HIV-1 based nucleic acids delivery systems, for example, in gene therapy. An HIV-2 based vector is packaged by the packaging cell lines, demonstrating that HIV-2 cell transformation vectors are packaged by the packaging cell line. HIV based vectors packaged by the high efficiency cell lines are shown to have anti-HIV activity per se.

CLM What is claimed is:

1. A cell transformation vector comprising a first HIV LTR, a second HIV LTR, and an HIV-2 packaging site nucleic acid between the two HIV LTRs and an HIV -1 gag protein, wherein at least one of the HIV LTRs is an HIV-2 LTR from HIV-2.sup.KR.
2. The vector of claim 1, wherein the vector further comprises a transcription cassette, which transcription cassette comprises a heterologous promoter which directs expression of a nucleic acid of interest, and further wherein the transcription cassette is located in one of the HIV LTRs.
3. The vector of claim 2, wherein the nucleic acid of interest encodes a ribozyme which specifically cleaves an HIV nucleic acid.
4. The vector of claim 3, wherein the promoter is a pol III promoter.
5. The vector of claim 4, wherein the first HIV LTR is an HIV-2.sup.KR 5'LTR and the second HIV LTR is an HIV-2.sup.KR 3'LTR.
6. A method of transforming a cell in vitro, comprising contacting the cell with the vector of claim 1.
7. The method of claim 6, wherein the method further comprises isolating the cell from a mammal prior to contacting the cell with the vector.
8. The method of claim 6, wherein the cell is a CD34.sup.+ stem cell.
9. A cell transformation vector comprising a first HIV LTR, a second HIV LTR, and an HIV-2 packaging site nucleic acid between the two HIV LTRs and an HIV -1 gag protein, wherein the vector further comprises an MPMV nucleic acid nuclear export subsequence.
10. The vector of claim 9, wherein the vector further comprises a transcription cassette, which transcription cassette comprises a

heterologous promoter which directs expression of a nucleic acid of interest, and further wherein the transcription cassette is located in one of the HIV LTRs.

11. The vector of claim 10, wherein the nucleic acid of interest encodes a ribozyme which specifically cleaves an HIV nucleic acid.

12. The vector of claim 11, wherein the promoter is a pol III promoter.

13. The vector of claim 12, wherein the first HIV LTR is an HIV-2 5'LTR and the second HIV LTR is an HIV-2 3'LTR.

14. A method of transforming a cell in vitro, comprising contacting the cell with the vector of claim 9.

15. The method of claim 14, wherein the method further comprises isolating the cell from a mammal prior to contacting the cell with the vector.

16. The method of claim 15, wherein the cell is a CD34.sup.+ stem cell.

17. A cell transformation vector comprising a first HIV LTR, a second HIV LTR, and an HIV-2 packaging site nucleic acid between the two HIV LTRs and an HIV-1 gag protein, wherein the vector further comprises a transcription cassette, which transcription cassette comprises a heterologous promoter which directs expression of a nucleic acid of interest, and further wherein the transcription cassette is located in one of the HIV LTRs.

18. The vector of claim 17, wherein the transcription cassette is located in the first HIV LTR, and wherein the vector further comprises a second transcription cassette located in the second HIV LTR.

19. The vector of claim 18, wherein the vector further comprises a third transcription cassette located between the first HIV LTR and the second HIV LTR.

20. The vector of claim 17, wherein the nucleic acid of interest encodes a ribozyme which specifically cleaves an HIV nucleic acid.

21. The vector of claim 17, wherein the promoter is a pol III promoter.

22. The vector of claim 17, wherein the promoter is a t-RNAval promoter.

23. The vector of claim 17, wherein the first HIV LTR is an HIV 5'LTR and the second HIV LTR is an HIV 3'LTR.

24. The vector of claim 23, wherein the HIV 5'LTR is an HIV-2 5'LTR and the HIV 3'LTR is an HIV-2 3'LTR.

25. The vector of claim 17, wherein the transcription cassette is located in a U3 region of at least one of the HIV LTRs.

26. The vector of claim 25, wherein the transcription cassette is located in a U3 region of the second HIV LTR which is an HIV-2 3'LTR.

27. The vector of claim 17, wherein the vector further comprises a VSV envelope protein.
28. The vector of claim 17, wherein at least one of the HIV LTRs is an HIV-2 LTR.
29. A method of transforming a cell in vitro, comprising contacting the cell with the vector of claim 17.
30. The method of claim 29, wherein the method further comprises isolating the cell from a mammal prior to contacting the cell with the vector.
31. The method of claim 29, wherein the cell is a CD34.sup.+ stem cell.
32. The method of claim 29, wherein the nucleic acid of interest encodes a ribozyme which specifically cleaves an HIV nucleic acid and wherein transforming the cell with the vector renders the cell resistant to HIV infection.
33. A cell transformation vector comprising an HIV 5'LTR, an HIV 3'LTR, and an HIV-2 packaging site nucleic acid between the two HIV LTRs, and viral particles comprising an HIV-1 gag protein, an env protein, and an HIV-1 vpr protein, wherein the HIV-1 gag protein is a p25 subproduct.
34. The vector of claim 33, wherein the HIV 5'LTR is an HIV-2 5'LTR and the HIV 3'LTR is an HIV-2 3'LTR.
35. The vector of claim 34, wherein the vector further comprises a transcription cassette, which transcription cassette comprises a heterologous promoter which directs expression of a nucleic acid of interest, and further wherein the transcription cassette is located in one of the HIV LTRs.
36. The vector of claim 35, wherein the nucleic acid of interest encodes a ribozyme which specifically cleaves an HIV nucleic acid.
37. The vector of claim 36, wherein the promoter is a pol III promoter.
38. The vector of claim 33, wherein the vector further comprises a VSV envelope protein.
39. A method of transforming a cell in vitro, comprising contacting the cell with the vector of claim 33.
40. The method of claim 39, wherein the method further comprises isolating the cell from a mammal prior to contacting the cell with the vector.
41. The method of claim 40, wherein the cell is a CD34.sup.+ stem cell.

L27 ANSWER 81 OF 96 MEDLINE on STN

94208520 Document Number: 94208520. PubMed ID: 8156990. Specific binding of HIV-1 nucleocapsid protein to PSI RNA in vitro requires N-terminal zinc finger and flanking basic amino acid residues. Dannull J; Surovoy A; Jung G; Moelling K. (Max-Planck-Institut fur Molekulare Genetik, Abteilung Schuster, Germany.) EMBO JOURNAL, (1994 Apr 1) 13 (7) 1525-33. Journal code: 8208664. ISSN: 0261-4189. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The nucleocapsid (NC) protein of human immunodeficiency virus HIV-1 (NCp7) is responsible for packaging the viral RNA by recognizing a packaging site (PSI) on the viral RNA genome. NCp7 is a molecule of 55 amino acids containing two zinc fingers, with only the first one being highly conserved among retroviruses. The first zinc finger is flanked by two basic amino acid clusters. Here we demonstrate that chemically synthesized NCp7 specifically binds to viral RNA containing the PSI using competitive filter binding assays. Deletion of the PSI from the RNA abrogates this effect. The 35 N-terminal amino acids of NCp7, comprising the first zinc finger, are sufficient for specific RNA binding. Chemically synthesized mutants of the first zinc finger demonstrate that the amino acid residues C-C-C/H-C/H are required for specific RNA binding and zinc coordination. Amino acid residues F16 and T24, but not K20, E21 and G22, located within this zinc finger, are essential for specific RNA binding as well. The second zinc finger cannot replace the first one. Furthermore, mutations in the basic amino acid residues flanking the first zinc finger demonstrate that R3, 7, 10, 29 and 32 but not K11, 14, 33 and 34 are also essential for specific binding. Specific binding to viral RNA is also observed with recombinant NCp15 and Pr55Gag. The results demonstrate for the first time specific interaction of a retroviral NC protein with its PSI RNA in vitro.

L27 ANSWER 82 OF 96 MEDLINE on STN

94047332 Document Number: 94047332. PubMed ID: 8230441. Specific binding of human immunodeficiency virus type 1 gag polyprotein and nucleocapsid protein to viral RNAs detected by RNA mobility shift assays. Berkowitz R D; Luban J; Goff S P. (Department of Biochemistry and Molecular Biophysics, College of Physicians and Surgeons, Columbia University, New York, New York 10032.) JOURNAL OF VIROLOGY, (1993 Dec) 67 (12) 7190-200. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Packaging of retroviral genomic RNA during virion assembly is thought to be mediated by specific interactions between the gag polyprotein and RNA sequences (often termed the psi or E region) near the 5' end of the genome. For many retroviruses, including human immunodeficiency virus type 1 (HIV-1), the portions of the gag protein and the RNA that are required for this interaction remain poorly defined. We have used an RNA gel mobility shift assay to measure the in vitro binding of purified glutathione S-transferase-HIV-1 gag fusion proteins to RNA riboprobes. Both the complete gag polyprotein and the nucleocapsid (NC) protein alone were found to bind specifically to an HIV-1 riboprobe. Either Cys-His box of NC could be removed without eliminating specific binding to the psi riboprobe, but portions of gag containing only the MA and CA proteins without NC did not bind to RNA. There were at least two binding sites in HIV-1 genomic RNA that bound to the gag polyprotein: one entirely 5' to gag and one entirely within gag. The

HIV-1 NC protein bound to riboprobes containing other retroviral psi sequences almost as well as to the HIV -1 psi riboprobe.

L27 ANSWER 87 OF 96 MEDLINE on STN

93278285 Document Number: 93278285. PubMed ID: 1304355.

Nucleocapsid zinc fingers detected in retroviruses: EXAFS studies of intact viruses and the solution-state structure of the nucleocapsid protein from HIV-1. Summers M F; Henderson L E; Chance M R; Bess J W Jr; South T L; Blake P R; Sagi I; Perez-Alvarado G; Sowder R C 3rd; Hare D R; +. (Department of Chemistry and Biochemistry, University of Maryland Baltimore County 21228.) PROTEIN SCIENCE, (1992 May) 1 (5) 563-74. Journal code: 9211750. ISSN: 0961-8368. Pub. country: United States. Language: English.

AB All retroviral nucleocapsid (NC) proteins contain one or two copies of an invariant 3Cys-1His array (CCHC = C-X2-C-X4-H-X4-C; C = Cys, H = His, X = variable amino acid) that are essential for RNA genome packaging and infectivity and have been proposed to function as zinc-binding domains. Although the arrays are capable of binding zinc in vitro, the physiological relevance of zinc coordination has not been firmly established. We have obtained zinc-edge extended X-ray absorption fine structure (EXAFS) spectra for intact retroviruses in order to determine if virus-bound zinc, which is present in quantities nearly stoichiometric with the CCHC arrays (Bess, J.W., Jr., Powell, P.J., Issaq, H.J., Schumack, L.J., Grimes, M.K., Henderson, L.E., & Arthur, L.O., 1992, J. Virol. 66, 840-847), exists in a unique coordination environment. The viral EXAFS spectra obtained are remarkably similar to the spectrum of a model CCHC zinc finger peptide with known 3Cys-1His zinc coordination structure. This finding, combined with other biochemical results, indicates that the majority of the viral zinc is coordinated to the NC CCHC arrays in mature retroviruses. Based on these findings, we have extended our NMR studies of the HIV-1 NC protein and have determined its three-dimensional solution-state structure. The CCHC arrays of HIV-1 NC exist as independently folded, noninteracting domains on a flexible polypeptide chain, with conservatively substituted aromatic residues forming hydrophobic patches on the zinc finger surfaces. These residues are essential for RNA genome recognition, and fluorescence measurements indicate that at least one residue (Trp37) participates directly in binding to nucleic acids in vitro. The NC is only the third HIV-1 protein to be structurally characterized, and the combined EXAFS, structural, and nucleic acid-binding results provide a basis for the rational design of new NC-targeted antiviral agents and vaccines for the control of AIDS.

L27 ANSWER 88 OF 96 MEDLINE on STN

93184722 Document Number: 93184722. PubMed ID: 8443588. Zinc- and sequence-dependent binding to nucleic acids by the N-terminal zinc finger of the HIV-1 nucleocapsid protein: NMR structure of the complex with the Psi-site analog, dACGCC. South T L; Summers M F. (Department of Chemistry and Biochemistry, University of Maryland Baltimore County 21228.) PROTEIN SCIENCE, (1993 Jan) 2 (1) 3-19. Journal code: 9211750. ISSN: 0961-8368. Pub. country: United States. Language: English.

AB The nucleic acid interactive properties of a synthetic peptide with sequence of the N-terminal CCHC zinc finger (CCHC = Cys-X2-Cys-X4-His-X4-Cys; X = variable amino acid) of the human immunodeficiency virus (HIV) nucleocapsid protein, Zn(HIV1-F1), have been studied by 1H NMR

spectroscopy. Titration of Zn(HIV1-F1) with oligodeoxyribonucleic acids containing different nucleotide sequences reveals, for the first time, sequence-dependent binding that requires the presence of at least one guanosine residue for tight complex formation. The dynamics of complex formation are sensitive to the nature of the residues adjacent to guanosine, with residues on the 3' side of guanosine having the largest influence. An oligodeoxyribonucleotide with sequence corresponding to a portion of the HIV-1 psi-packaging signal, d(ACGCC), forms a relatively tight complex with Zn(HIV1-F1) ($K_d = 5 \times 10^{-6}$ M). Two-dimensional nuclear Overhauser effect (NOESY) data indicate that the bound nucleic acid exists predominantly in a single-stranded, A-helical conformation, and the presence of more than a dozen intermolecular NOE cross peaks enabled three-dimensional modeling of the complex. The nucleic acid binds within a hydrophobic cleft on the peptide surface. This hydrophobic cleft is defined by the side chains of residues Val1, Phe4, Ile12, and Ala13. Backbone amide protons of Phe4 and Ala13 and the backbone carbonyl oxygen of Lys2 that lie within this cleft appear to form hydrogen bonds with the guanosine O6 and NH atoms, respectively. In addition, the positively charged side chain of Arg14 is ideally positioned for electrostatic interactions with the phosphodiester backbone of the nucleic acid. The structural findings provide a rationalization for the general conservation of these hydrophobic and basic residues in CCHC zinc fingers, and are consistent with site-directed mutagenesis results that implicate these residues as direct participants in viral genome recognition.

L27 ANSWER 89 OF 96 MEDLINE on STN
93156817 Document Number: 93156817. PubMed ID: 8429889. Inhibition of HIV-1 infectivity by zinc-ejecting aromatic C-nitroso compounds. Rice W G; Schaeffer C A; Harten B; Villinger F; South T L; Summers M F; Henderson L E; Bess J W Jr; Arthur L O; McDougal J S; +. (Laboratory of Antiviral Drug Mechanisms, Program Resources Inc./DynCorp., National Cancer Institute-Frederick Cancer Research and Development Center, Maryland 21702.) NATURE, (1993 Feb 4) 361 (6411) 473-5. Journal code: 0410462. ISSN: 0028-0836. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Retroviral nucleocapsid and gag-precursor proteins from all known strains of retroviruses contain one or two copies of an invariant sequence, Cys-X2-Cys-X4-His-X4-Cys, that is populated with zinc in mature particles. Modification of cysteine or histidine residues results in defective packaging of genomic viral RNA and formation of non-infectious particles, making these structures potentially attractive targets for antiviral therapy. We recently reported that aromatic C-nitroso ligands of poly(ADP-ribose) polymerase preferentially destabilize one of the two (Cys-X2-Cys-X28-His-X2-Cys) zinc-fingers with concomitant loss of enzymatic activity, coincidental with selective cytotoxic action of the C-nitroso substituted ligands on cancer cells. Based on the occurrence of (3Cys, 1His) zinc-binding sites in both retroviral nucleocapsid and gag proteins and in poly(ADP-ribose) polymerase, we reasoned that the C-nitroso compounds may also have antiretroviral effects. We show here that two such compounds, 3-nitrosobenzamide and 6-nitroso-1,2-benzopyrone, inhibit infection of human immunodeficiency virus HIV-1 in human lymphocytes and also eject zinc from isoalted HIV-1 nucleocapsid zinc fingers and from intact HIV-1 virions. Thus the design of zinc-ejecting agents that target retroviral zinc fingers represents a new approach to the chemotherapy of AIDS.

L27 ANSWER 93 OF 96 MEDLINE on STN
92263762 Document Number: 92263762. PubMed ID: 1585635. RNA

packaging signal of human immunodeficiency virus type 1. Hayashi T; Shioda T; Iwakura Y; Shibuta H. (Department of Viral Infection, University of Tokyo, Japan.) VIROLOGY, (1992 Jun) 188 (2) 590-9. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

- AB Cells infected with a recombinant vaccinia virus carrying the gag and pol regions of the human immunodeficiency virus type 1 genome (Vac-gag/pol) released human immunodeficiency virus (HIV)-like particles containing HIV-specific RNA. However, cells infected with another recombinant vaccinia, Vac-gag/pol-dP, derived through the deletion of an 85-base region (nucleotide positions 679-763) of the HIV genome between the primer binding site and the gag initiation codon of Vac-gag/pol, produced HIV-like particles devoid of the HIV-specific RNA. This 85-base deletion was suggested to cause the collapse of a stable stem-loop structure of 46 bases (751-796) around the gag initiation codon. To examine the role of the stem-loop structure in the packaging of RNAs, we constructed a vaccinia vector plasmid that carried this 46-base sequence followed by the Sendai virus nucleocapsid (NP) gene. When both Vac-gag/pol-dP and this plasmid were introduced into cells, HIV-like particles released from the cells contained the NP gene RNA. However, another vaccinia vector plasmid, which carried the 46-base sequence in the midst of the NP gene, could not supply RNA for incorporation into HIV-like particles. Computer analysis of this plasmid sequence suggested that the 46-base sequence cannot form the stem-loop structure. These findings suggest that the stem-loop structure formed by the 46-base sequence is crucial as a packaging signal.